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RESEARCH

Cattle Growth Promoter Adversely Affects Reproductive System of the Rat

Trenbolone acetate is a synthetic steroid hormone used to promote growth in beef cattle. It is quickly metabolized to 17-β-trenbolone, an active androgen (a male sex hormone) known to induce male sex characteristics and infertility in female fish. Trenbolone is also one of the metabolites excreted by cattle into the environment. A paper soon to be published in *Toxicological Sciences* by Dr. Vickie Wilson, Dr. Earl Gray, Joseph Ostby, and Christy Lambright of the National Health and Environmental Effects Research Laboratory (NHEERL), Reproductive Toxicology Division (RTD), shows that trenbolone mimicked the male sex hormones in rats. Trenbolone may also cause environmental or human risks as an endocrine disruptor. The European Union has banned the import of beef treated with hormones, in part because trenbolone can persist in the environment for relatively long periods of time in an active form.

The RTD studies used an *in vitro* cell-based assay to identify possible androgenic activity of feedlot effluent taken from a concentrated animal feedlot operation. These assays detected significant androgenic activity in the sample, but whether that hormonal activity was due solely to trenbolone is unclear. When tested in rats, trenbolone had activity that differed depending on the specific tissue examined in castrated males, and it caused reproductive tract malformations in female offspring of rats treated during pregnancy. Both observations show that trenbolone has activity that is similar, but not identical, to that of testosterone.

Further work is in progress to investigate long-term effects of this chemical on reproduction and development of animals in both the laboratory and the field.

Dietary Folate Deficiency Increases Arsenic Genotoxicity in Mice

An important need in cancer risk assessment is to identify subpopulations of individuals with unusually high sensitivities to the damaging effects of toxic chemicals in the environment. Individuals with lifestage- or lifestyle-related nutritional deficits of the B vitamin, folate, including many pregnant women, adolescents, and elderly subjects, comprise large segments of the American population. This condition has also been linked to widespread public health problems in developing countries. Research in the Environmental Carcinogenesis Division (ECD) showed that the combined effects from dietary folate deficiency and exposure to arsenic in mice results in increased genotoxicity.

Dr. James Allen and Barbara Collins of ECD and Elena McDorman, a graduate student in the Curriculum in Toxicology, University of North Carolina at Chapel Hill, used a mouse model to evaluate whether dietary folate deficiency predisposes toward greater genotoxic effects from exposure to arsenic, a contaminant of drinking water and a known human carcinogen. They showed that folate deficiency, coupled with high doses of arsenic, significantly increases arsenic exposure effects to cause chromosome damage detectable in blood cells. Induction of chromosome abnormalities is a potentially significant carcinogenic mode of action for arsenic. Thus, dietary folate deficiency may define susceptible populations at increased risk of carcinogenesis and other genetic maladies from exposure to arsenic. [Wiley InterScience online, July 24, 2002; *Environmental and Molecular Mutagenesis*, 2002, 40(1):71-77].

Genomics Used to Study Health Effects of PM Air Pollution in Rats

Particulate matter (PM), is a type of air pollution consisting of complex and varying mixtures of particles (gaseous combustion by-products, fine solids, metals, soil dust, ashes, soot, pollens, etc.) suspended in the air. There are many PM sources, including factory and utility smokestacks, vehicle exhaust, wood burning, mining,

construction activities, and cigarette smoking. PM air pollution is particularly harmful to people with heart and lung diseases, but the biochemical basis for the adverse health effects is not clear. Drs. Srikanth Nadadur and Urmila P. Kodavanti in the Experimental Toxicology Division used modern genomics technologies to understand the toxic effects of PM on rat lung.

Genomics is the current-state-of-the art molecular biology technology used to scan gene messages or messenger RNA (mRNA) molecules for thousands of genes in a single experiment. Any stress, including physical stressors such as heat and cold, sends signals from the cell membrane to the nucleus. The nucleus responds by turning on the synthesis of mRNA, which is then translated into specific proteins or enzymes to protect against the stress. Measuring the levels of mRNA indicates the active state of the gene of interest. Based on the involvement of multiple genes in the pathogenesis of various lung and heart diseases, Dr. Nadadur constructed a tissue-specific gene array to evaluate changes in the levels of messages for multiple genes and to understand their role in mediating the toxic effects.

Laboratory rats were exposed to an aqueous extract of a PM combustion source, residual oil fly ash (ROFA), and to two of its major toxic metals, nickel and vanadium. The gene expression levels were monitored in the rat lung tissue 3 and 24 hours after exposure. Based on the number of genes with increased expression (more messages), ROFA-exposed rat lungs showed increased toxic effects, compared to those exposed to either nickel and vanadium alone. This increased toxicity may result from interaction among various constituents in ROFA, compared to that from the individual metals. Understanding the interaction of toxicants at the gene message level is very important in developing molecular toxicology models.

These results will be used to understand the role of altered gene expression in the mechanism of action and, ultimately, the pathology of the lung. Data generated

from animal studies will then be extrapolated to understand the effects on humans. [*Journal of Toxicology and Environmental Health, Part A*, 2002, 65:1333-1350].

Regional Differences Are Critical for Wetland Assessments

Scientists associated with the Western Ecology Division (WED) and Pennsylvania State University's Cooperative Wetlands Center have published an article evaluating the transferability of new wetland assessment models from one geographic region to another. They found that attempts to transfer hydrogeomorphic (HGM) models from one region to another can lead to serious errors in interpretation, especially in wetlands supported by surface water.

The HGM approach to wetland classification and assessment is becoming widely used. Rather than simply categorizing wetland plants or structural features such as substrate characteristics, as other classification systems do, HGM is based on features important to the formation and maintenance of wetlands. The HGM approach involves understanding the source of a wetland's water and how that water interacts with the geomorphology of the site–its shape, soils, and geology. It takes a lot of work, however, to develop and test a version of HGM appropriate for use in a particular region. The effort needed could be decreased if scientists understood what factors were most important to the transferability of a regional version to other regions.

By examining the comparability of hydrologic characteristics of three hydrogeomorphic classes of wetlands found both in Pennsylvania's Ridge and Valley area and in Oregon's Willamette Valley, Dr. Mary Kentula of WED and colleagues from Penn State and Dynamac Corporation--Environmental Services investigated the transferability problem. They found that the key to using the HGM approach to wetland classification and assessment is understanding how the factors important to the ecological function of the system operate in the particular region. For example, slope wetlands (groundwater-driven) were found to have similar hydrologic characteristics in

Pennsylvania and Oregon, but floodplain wetlands in the two areas were not comparable. Regional characteristics, especially climate and soil type, are important in explaining the differences. For example, the lengthy rainy season that occurs each fall and winter in western Oregon means that the compacted clay soils of the floodplain sites are constantly anaerobic for much of the year, favoring denitrification and accumulation of organic matter during the wet months. The more variable precipitation throughout the year in Pennsylvania means that the coarse floodplain soils there alternate between wet and dry conditions. These variable water levels cause fluctuations between aerobic and anaerobic conditions in the soil, favoring nutrient cycling. Thus, wetlands in different regions do not necessarily function in the same way, even if they are in the same HGM class. [*Environmental Management, 2002,* 30(2):265-278].

Androstenedione Does Not Masculinize Female Fish Downstream of Paper Mill

As early as 1978, masculinized female mosquitofish were found in several rivers, including the Fenholloway, in central Florida. Although researchers proposed a linkage to components of pulp and paper mill discharges that acted as androgen receptor agonists (male sex hormones), there were no supporting data. With recent growing interest in endocrine-disrupting chemicals in the environment, there is renewed attention to masculinized fish downstream of pulp and paper mill discharges. Non-EPA studies found androgen receptor agonists in water samples from the Fenholloway River and suggested that androstenedione (4-androsten-3,17-dione), which has moderate androgenic activity, may be the hormone responsible. A recent NHEERL paper, however, showed that androstenedione is not a major contributor to androgenic activity in waters of the Fenholloway River. NHEERL co-authors include Dr. Gary Ankley, Elizabeth Durhan, Brian Butterworth, and Douglas Kuehl of the Mid-Continent Ecology Division and Dr. Earl Gray, Dr. Vickie Wilson, and Christy Lambright of the Reproductive Toxicology Division.

These researchers and University of Florida colleagues studied water samples collected downstream of a pulp and paper mill on the Fenholloway River between May 1999 and May 2001 and measured the androgenic activity in river water extracts and fractions by several methods. Androstenedione was found to be present at a low concentration (120 ng/l), but did not contribute significantly to *in vitro* androgenic activity. Further research will be conducted to identify which chemicals are responsible for androgenicity in Fenholloway water downstream of the pulp and paper mill effluent. [*Environmental Toxicology and Chemistry*, 2002, 21:1973-1976].

Diatoms Used to Diagnose Causes of Stream Impairment

As watersheds are converted from forests to agricultural and urban environments, streams draining these watersheds are impacted by human activities. Multi-metric indices of biotic integrity (IBI), the most common approach to biological monitoring of streams, are individual biological measurements that clearly relate to specific environmental stressors. Using an IBI based on diatoms, a type of algae common to all aquatic ecosystems, NHEERL researchers and colleagues assessed the quality of 272 Mid-Atlantic streams. They concluded that 4% of the stream length studied was in excellent condition; 21% in good condition; 56% in fair condition; and 19% in poor condition.

As a part of EPA's Environmental Monitoring and Assessment Program, Drs. Brian Hill of the Mid-Continent Ecology Division and Philip Kaufmann of the Western Ecology Division are among the authors of a paper describing this research to be published in *Ecological Indicators* in late 2002 or early 2003. The diatom characteristics and the IBI used were responsive to stressor gradients associated with nutrient enrichment, acidification, siltation, and riparian zone agriculture.

The Clean Water Act (CWA) requires states, territories, tribes, and interstate commissions to assess the conditions of their waters and the extent to which these

waters meet water quality standards and support designated uses. The most recent national water quality inventory, in 2000, indicates that 45% of total stream length in the United States is either impaired or threatened with impairment. The diatom IBI is recommended as a useful measure of ecological conditions in streams because it provides insight into the causes of impairment. An IBI also provides a quick assessment of the overall condition of a stream that is easily understood by non-technical resource managers, while the individual metrics provide insight into the causes of impairment. Use of the IBI and established assessment criteria gives a realistic and defensible assessment of the ecological condition of streams and a benchmark against which future studies may be compared.

PUBLICATIONS

Criteria Described for Evaluating PAH Contaminants in Sediments

Western Ecology Division (WED) scientists contributed a chapter to *Chemicals in the Environment: Fate, Impacts, and Remediation*, American Chemical Society Symposium Series 806, edited by R. L. Lipnick et al., and published by Oxford University Press in 2001. In Chapter 14, "Development and Application of Equilibrium Partitioning Sediment Guidelines in the Assessment of Sediment PAH Contamination," Drs. Robert Ozretich and David Young of WED and Dr. D. B. Chadwick of the U.S. Navy/San Diego describe the 15-year process taken by EPA to develop guidelines for evaluating polynuclear aromatic hydrocarbon (PAHs) in marine and freshwater sediments.

This chapter discusses how procedures and data requirements used by EPA in developing water quality criteria (WQC) were then applied to developing guidelines for the protection of benthic organisms in sediments. The biophysical model underlying these guidelines incorporates four toxicity principles: Only freely dissolved contaminants are toxic; robust relationships exist between a chemical's characteristics

and its toxicity; non-ionic PAHs are additive in their effects; and PAHs accumulate in the lipids of the organisms, causing abnormal behavior or death.

The proposed guidelines were then applied to sediment and interstitial water (water in spaces between sediment particles) at 10 sites in Sinclair Inlet near the Puget Sound Naval Shipyard at Bremerton, WA. Using PAH concentrations found in the sediments alone, seven of the sites would have been classified as “Sites of Concern”; whereas, if PAH concentrations were based on those found in interstitial water alone, none of the sites would have been classified as such. Another EPA procedure is used to reconcile these seemingly contradictory conclusions. It exploits the much greater understanding of the biology-chemistry-physics interplay within sediments that has accrued since the guidelines were first developed.